ENZYMES IN ONTOGENESIS (ORTHOPTERA)

XIX. Protyrosinase and Morphological Integrity of Grasshopper Eggs [1]

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Although protyrosinase has been found in extracts of grasshopper eggs, no evidence for its existence within the intact egg has been presented. In view of the possibility that the very process of extraction might inactivate the enzyme, it seems desirable to examine the relation of protyrosinase to morphological integrity. It should be possible to perform such a test by subjecting eggs to one of those treatments which cause the activation of extracted protyrosinase. An increased rate of oxygen uptake and the appearance of melanin in the intact egg should then indicate that protyrosinase had been present before its transition into tyrosinase. This paper deals with results of experiments showing the occurrence of protyrosinase within the intact egg of a grasshopper, *Melanoplus differentialis* (Thomas).

The data which are graphically illustrated in the accompanying figure were obtained from recordings of a Warburg apparatus operated at 24.9° C. The time course of oxygen uptake was plotted for groups of 100 intact eggs which had just previously been heated for five minutes in water kept at certain indicated temperatures. The rates of oxygen uptake of diapause eggs heated between 62° to 85° C. remained constant through the first 100 cu.mm. but declined as a limiting volume of 225 to 230 cu.mm. was approached. However, the rates of oxygen uptake of eggs which had been exposed to temperatures below 50° C. were constant. Relative values for the velocity of oxygen uptake may thus be given by the reciprocal of the time in minutes for the utilization of the initial 100 cu.mm. of oxygen. When these values are compared, a complex temperature effect is found (see figure). It is proposed to interpret this effect according to the properties and occurrence of protyrosinase.

If an egg extract containing protyrosinase is heated for five minutes at temperatures between 60° and 85° C., a tyrosinase is formed (Bodine

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and Allen, 1938). Heating seems to affect the stability of both protyrosinase and tyrosinase. With ascending temperature the former is activated, while the latter is destroyed. Consequently, the tyrosinase activity of an extract increases from 60° to 75° but declines from 75°

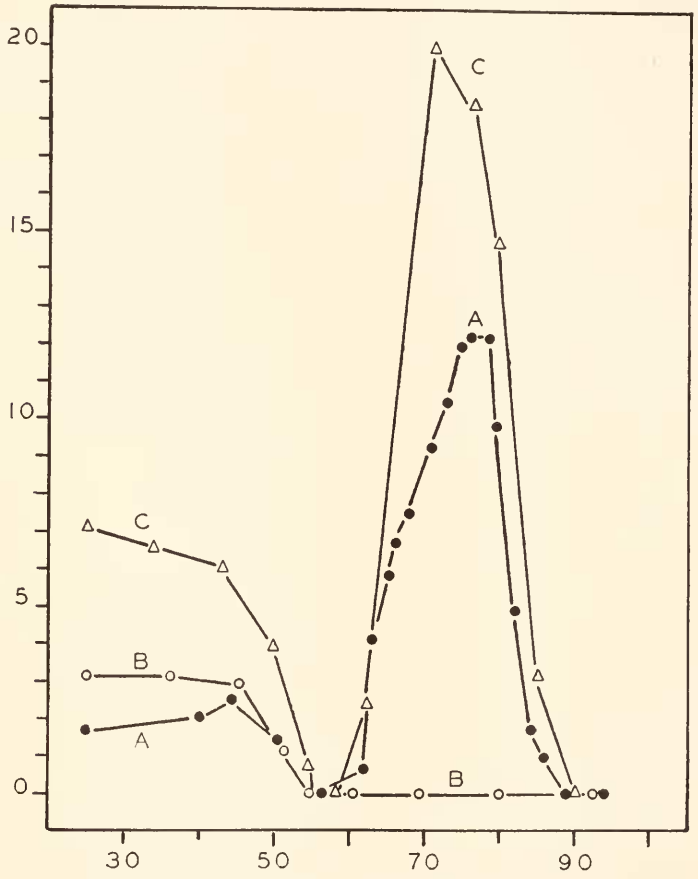

FIG. 1. The effect of heat treatment on the oxygen uptake of grasshopper eggs in various stages of development. Ordinate, reciprocal of the time in minutes for the uptake of 100 cu.mm. of oxygen multiplied by 1000; abscissa, temperatures in °C. to which eggs were exposed for five minutes. Curve *B*, 7-day eggs (prediapause); curve *A*, 60-day eggs (diapause); curve *C*, eggs 3 days post-diapause.

to 90°. A similar differential effect of heat is found for the velocity of oxygen uptake of intact diapause or post diapause eggs (see figure, curve *A* and *C*). Since protyrosinase and a naturally occurring substrate can be extracted from eggs of these stages (Bodine, Allen, and

Boell, 1937), it appears that the increased velocity of oxygen uptake of the intact egg must be due to the heat-induced enzymic oxidation of the native substrate. Curve *C* is presumably higher than curve *A,* because in post diapause there is more native substrate than in diapause (Bodine, Allen, and Boell, 1937).

The latter interpretation also seems to be supported by the eventual formation of melanin, by the low value for the "respiratory, quotient," and by the sensitivity to cyanide. Diapause eggs, which six hours previously had been heated between 62° to 84° C., changed from a pale lemon yellow to a dark olive-green color. Upon dissection it seemed that the darker color was due to the presence of a brown pigment—melanin—located in the "liquid-filled space" (Slifer, 1937) between the serosa and cuticle. Similar eggs heated below 62° and above 84° C. remained a lemon yellow, because their protyrosinase supposedly had either not been activated or else had been destroyed. From measurements of the oxygen uptake and carbon dioxide production performed according to the indirect method of Warburg (Dixon, 1934), an R.Q. of 0.1 to 0.2 was found for eggs that had been heated at 75°. Such a value is to be expected during the production of melanin (Raper, 1928). Potassium cyanide in a concentration of 0.01 M abolished the oxygen uptake produced by heat activation. These properties are usually considered to be characteristic of a tyrosinase reaction.

Since protyrosinase has not been found in extracts of eggs younger than eight to nine days of age (Bodine, Allen, and Boell, 1937), one should not expect an increased velocity of oxygen uptake for seven-day eggs that have been exposed to those various degrees of heat sufficient for activating protyrosinase. The occurrence of such a phenomenon would serve essentially as a control experiment for the heat treatment of those eggs containing protyrosinase (see figure, curve *B*). The respiratory processes of prediapause and diapause eggs are evidently susceptible to the effects of heat. Perhaps the normally working respiratory enzymes are destroyed at 56° C. (see figure). If such be the case, it may then be supposed that the portion of the curve for diapause or post-diapause eggs between 62° and 85° C. pertains entirely to the activation of protyrosinase and the destruction of tyrosinase.

The addition of an activator followed by the formation of an enzyme presumably should indicate through cause and effect relations that a proenzyme had once been present. It therefore seems that heat treatment has demonstrated the occurrence of protyrosinase as a constituent of diapause and post-diapause grasshopper eggs. This demonstration of protyrosinase seems to be independent of the trituration and dilution

inherent to an extraction process. Thus it appears that protyrosinase exists within the intact grasshopper egg and that this protyrosinase does not lose characteristic properties as a result of extraction. Moreover, these deductions on the occurrence of the inactive rather than the active enzyme would lead to the conclusion that oxidations coupled with a tyrosinase reaction (Allen and Bodine, 1940) can hardly be expected to complement the respiratory processes of these eggs. Although extracted protyrosinase can be activated by an oil native to these eggs (Bodine, Allen, and Boell, 1937), this lipide is probably bound to various proteins or isolated in such a way that it is inaccessible to the protyrosinase of intact eggs (Bodine and Carlson, 1940).

Summary

Protyrosinase occurs in the intact egg of the grasshopper, *Melanoplus differentialis,* and shows properties similar to those for extracts prepared by trituration of the eggs. Moreover, it seems that protyrosinase, as a naturally occurring entity, is not an artefact produced by extraction procedures.

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